IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of.:			
	Irit SAGI et al		
Serial No.:	10/551,715		
Filed:	July 20, 2006	Group Art Unit:	1652
For:	ANTIBODIES AND PHARMACEUTICAL COMPOSITIONS CONTAINING SAME USEFUL FOR INHIBITING ACTIVITY OF METALLOPROTEINS		
		Attorney Docket:	**29993**
Examiner:	Mohammad Y. Meah		

Mail Stop Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313

DECLARATION OF IRIT SAGI
UNDER 37 CFR 1.132

I am presently employed as a researcher at the Weizmann Institute of Science, Israel, Department of Structural Biology, where I am a professor of Biophysics. I received my Ph.D. degree from the Georgetown University in 1992, and worked as a post-doctoral fellow in the laboratory of Prof. Ada Yonath at Weizmann institute of Science, Israel and Max Planck Institute, Germany .

Since the beginning of my career, I have published more than 50 scientific articles in highly regarded journals and books, and have presented my achievements at many international scientific conferences.

I was awarded several research prizes including the Inventor of the year YEDA award.

I am an inventor of the above-identified application.

I have read the Official action issued with respect to the above-identified application.

In the Official Action, the Examiner rejected Claims 7-14, 35 and 42 under 35 U.S.C. § 112 first paragraph for not being enabled.

Since the date of filing, experiments performed under my supervision and in my laboratory have clearly illustrated that Zn-TCPP may be used as a hapten for generating monoclonal antibodies against gelatinase B (MMP-9) and MMP-14.

Specifically, a monoclonal antibody against ZnTCPP was generated that displayed a high affinity (Kd 0.01 μM) toward its immunizing hapten based on a competitive ELISA screen. This antibody was shown to bind to gelatinase B (MMP-9) catalytic domain and gelatinase A (MMP-2) – see Figures 2A-C attached.

To establish that binding occurred through direct interaction with the active site, mAbs were analyzed for their ability to bind Pro-MMP-2 and Pro-MMP-9. In the latent enzymes the pro domain structure shields the catalytic cleft. Hence, blocking of the active site by the pro-domain structure should prevent mAbs binding, providing it recognizes the histidine zinc motif within the active site. Under the same conditions, no binding to the pro enzymes was detected (Figure 2B). This mode of binding to active MMP-2 but not to Pro-MMP-2 was further challenged in an *in vivo* like environment with full length native MMP-2 secreted by human fibrosarcoma (HT1080) cell cultures. Immunoprecipitation of HT1080 conditioned medium with anti-CoTCPP antibody followed by western blot analysis showed binding to active but not Pro-MMP-2 (Figure 2C).

These results demonstrate that the monoclonal antibody raised against ZnTCPP cross reacted with MMP-2 and MMP-9 and recently MMP-14. Exposure of the active site cleft was essential for antibody binding, confirming that the antibody raised against ZnTCPP interacted directly with the active sites of MMP-2/9/14.

I hereby declare that all the statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and the such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Date: 7/8/2008

Prof. Irit Sagi
Enclosure:
CV of Prof. Irit Sagi

BIOGRAPHICAL SKETCH

NAME	POSITION TITLE
Irit Sagi, PhD	Associate Professor

INSTITUTION AND LOCATION	DEGREE	YEAR(s)	FIELD OF STUDY
American University, Washington DC	BSc	1989	Physical Chemistry
Georgetown University, Washington DC	PhD	1992	Bio-inorganic Chemistry
Weizmann Inst. of Science, Rehovot, Israel	Post Doc	1992-1994	Structural Biology
Max-Planck Inst. for Mol. Biol., Berlin, Germany	Post Doc	1994-1997	Structural Biology

POSITIONS

2005 - 2006 Visiting Scientist at Novartis Institutes for BioMedical Research (NIBR), Cambridge, MA
2005 - 2006 Visiting Scientist at Harvard University, The Rowland Institute, Cambridge, MA
2003 - present Associate Professor, Department of Structural Biology, The Weizmann Institute of Science, Rehovot, Israel
1997 - 2003 Assistant Professor, Department of Structural Biology, The Weizmann Institute of Science, Rehovot, Israel

Awards and Activities (partial list)

2007 Associate editor Wiley Encyclopedia of Analytical Chemistry.
2006 Inventor of the year award YEDA Ltd.
2005 Professorial chair of the Clotilde and Mauricio Pontecorvo Cathedra for Interdisciplinary Research at The Weizmann Institute of Science.
2005 Member of the scientific board of SESAME synchrotron.
2004 Member of the scientific review panel of the Brookhaven National Laboratory New-York synchrotron.
2003 Scientific Council Prize Weizmann Institute of Science.
2000 Elected member of the American Society for Biochemistry and Molecular Biology (ASBMB)
2000 Jakubskind-Cymerman research prize. The Weizmann Institute of Science, Israel.

PEER-REVIEWED PUBLICATIONS

1. Sagi, I., Wirt, M.D., Chen, E. Frisbie, S., Chance, M.R. (1990) Structure of an intermediate of coenzyme B_{12} catalysis by EXAFS: Co(II) B_{12}. Journal of the American Chemical Society 112, 8639-8644.
2. Wirt, M.D., Sagi, I., Chen, E., Frisbie, S., Chance, M.R.. (1991) Geometric conformations of intermediates of B_{12} catalysis by X-ray edge spectroscopy: Co(I) B_{12}, Co(II) B_{12}, and base off adenosylcobalamin. Journal of the American Chemical Society 113, 5299-5304.
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4. Sagi, I., Chance, M.R. (1992) The extent of trans effects in non-alkylcobalamins: steric effects control the Co-N distance to 5'6 dimethylbenzimidazole. Journal of the American Chemical Society 114, 8061-8066.
5. Wirt, M.D., Sagi, I., Chance, M.R. (1992) Formation of square planar Co(I) B_{12} intermediates: implications for enzymatic catalysis. Biophysical Journal 63, 412-417.
6. Chance, M.R., Sagi, I., Wirt, M.D., Frisbie, S.M., Scheuring, E., Chen, E., Bess, J.W., Henderson, L.E., Arthur, L.O., South, T.L., Perez-Alvarado, G., Summers, M.F. (1992) EXAFS studies of retrovirus: equine infectious anemia virus cysteine arrays are coordinated to zinc. Proceedings of the National Academy of Sciences USA 89, 10041-10045.
7. Scheuring, E., Sagi, I., Chance, M.R. (1994) Sulfur-containing cobalamins: X-ray absorption spectroscopic characterization. Biochemistry 33, 6310-6315.
8. Franceschi, F., Sagi, I., Boddeker, N., Evers, E., Paulke, C., Hacenbank, R., Laschever, M., Glotz, C., Piefke, J., Musiing, J., Weinstein, S., Yonath, A. (1994) Crystallographic, biochemical and genetic studies on halophilic ribosomes system and applications. Systems & Applied Microbiology 16, 697-705.
9. Sagi, I., Weinrich, V., Levin, I., Glotz, C., Laschever, M., Melamud, M., Franceschi, F., Weinstein, S., Yonath, A. (1995) Crystallography in ribosomes: attempts at decorating the ribosomal surface. Biophysical Chemistry 55, 31-41.
10. Schlunzen, F., Hansen, H.A.S., Thygesen, J., Volkmann, N., Levine, I., Harmes, J., Bartels, H., Bashan, A., Berkovitch-Yellin, Z., Sagi, I., Franceschi, F., Geva, M., Weinstein, S., Agmon, I., Boddeker, N., Morlang, S., Sharon, R., Peretz, M., Weinrich, V., Yonath, A. (1995)
11. A Milestone in ribosomal crystallography: the construction of preliminary electron density maps at intermediate resolution. Biochemistry & Cell Biology 73, 739-745.
12. Sagi, I., Hochman, Y., Bunker, G., Carmeli, S., Carmeli, C. (1998) Structure function relationship of vanadate bound to a single site in chloroplast CF1-ATPase as determined by X-ray Absorption. Photosynthesis Research 57, 275-285.
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16. Ring, S., Pedersen, H.B., Heber, O., Rappaport, M.L., Witte, P.D., Bhushan, K.G., Altstein, N., Rudich, Y., Sagi, I., Zajfman, D. (2000) Fourier transform time-of flight mass spectrometry in an electrostatic ion beam trap. Analytical Chemistry 72, 4041-4046.
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20. Henn, A., Medalia, O., Shi, S., Steinberg, M., Franceschi, F., Sagi, I. (2001) Visualization of unwinding activity of duplex RNA by dbpA, a DEAD Box helicase, at single molecule resolution by atomic force microscopy. Proceedings of the National Academy of Sciences USA 98, 5007-5012.
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42. Noy, D., Solomonov, I., Sinkevich, O, Arad, A., Kjaer, K., Sagi, I. (2008) Zinc-amyloid β interactions on a millisecond time-scale stabilize non-fibrillar Alzheimer related species. Journal of American Chemical Society, 130,1376-1383.

43. Lewis, K.C., Selzer, T., Shahar, C., Sagi, I. (2008) Novel inhibition of pectin methyl esterase activity by green tea catechins" (2008), Phytochemistry, In press.

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APPENDIX

Legend for Figures

FIGs. 1A-D are schematic representations of the molecular structure of Co/ZnTCPP - [meso-Tetrakis (4- carboxyphenyl)-porphyrinato] cobalt/zinc (II) (Figures 1A-B, Imisdp - [2-(2-minoethylcarbomoyl)-ethoxymethyl] -tris-[2-(N-(3-imidazol-1-yl-propyl)) -ethoxymethyl]methane, and the conserved zinc-protein ligation at the catalytic zinc site in MMPs.

FIGs. 1E-H are three dimensional schemes of the structures displayed in Figures 1A-D. Note, the ZnTCPP retains planar conformation while the CoTCPP exhibit a distorted microcycle conformation.

FIGs. 2A-C are western blot images showing the ability of mouse IgG - Agarose immobilized mAbs to pull down recombinant MMP-2 catalytic domain (MMP-2cat) or Pro-MMP-2 and Pro-MMP-9 from solution. Antibodies used for each experiment are 6C6 (immunizing hapten = Imisdp, see Figure 1C for structure), 13E11 (immunizing hapten = CoTCPP), and 13E15 (immunizing hapten = ZnTCPP). Figure 2A - MMP-2cat (2μg) was incubated with anti-mouse IgG - Agarose (cntl, lane1) or anti CoTCPP, ZnTCPP and Imisdp mAb (10μg) -anti-mouse IgG – Agarose for 2 hr at 20°C, immunoprecipitates (lane 2,3,5) were centrifuged and washed three times, separated on SDS/PAGE gel and visualized by Coomassie-staining. Figure 2B - Pro-MMP-2, Pro-MMP-9 were incubated with mAbs-anti-mouse IgG - Agarose in the same manner as in A. Immunoprecipitates (lane 2,4,6 left and 1,3,5 right) and unbound fraction (lane 1,3,5 left and 2,4,6 right) were separated on SDS/PAGE gel and visualized by Coomassie-staining. Figure 2C - conditioned medium of HT1080 cells that either underwent activation with APMA (left) or did not (right), was immunoprecipitated with anti CoTCPP mAb and analyzed by western blot with specific antibodies against MMP-2.